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Genetic Basis of Sensitivity in Sweet Corn to Tembotrione

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Tembotrione inhibits 4-hydroxyphenyl-pyruvate-dioxygenase (HPPD) and was recently registered for use in all types of corn. Some sweet corn hybrids are killed by tembotrione, yet a mechanistic understanding of sensitivity has not been reported. Sensitivity of mesotrione, another HPPD-inhibitor, is conditioned by a single allele. Two hypotheses were tested: (1) response to tembotrione and mesotrione are conditioned by alleles at the same or closely linked loci and (2) the extent of early-season injury from tembotrione and mesotrione is similar on hybrids. The first hypothesis was tested by comparing responses to tembotrione and mesotrione in 136 $F_{3:5}$ families derived from a cross of mesotrione-sensitive and mesotrione-tolerant sweet corn inbreds. F_3 families cosegregated for responses to tembotrione and mesotrione: 94% of the families had the same response to both herbicides. Thus, the same gene or very closely linked genes condition response to both herbicides. On the basis of chi-square goodness of fit tests, responses of families to tembotrione fit a 3 : 2 : 3 sensitive : segregating : tolerant ratio ($P = 0.24$), which would be expected if sensitivity to tembotrione was conditioned by a single recessive allele. The second hypothesis was tested in six field experiments by quantifying the extent of early-season injury to 249 sweet corn hybrids 1 wk after treatment (WAT) of tembotrione (184 g ha^{-1}) or mesotrione (210 g ha^{-1}). One hundred ninety-three hybrids were tolerant to both herbicides. Seven sensitive hybrids that were severely injured by both herbicides 1 WAT differed in their response 3 to 4 WAT; sensitive hybrids treated with mesotrione had apparently resumed normal growth, whereas those treated with tembotrione died. Conversely, hybrids with intermediate levels of injury (> 10 to 50%) 1 WAT with mesotrione had no visual symptoms of injury from applications of tembotrione. Despite the common genetic basis for response to mesotrione and tembotrione, hybrids with sensitive or intermediate responses to mesotrione did not have identical responses to tembotrione.

Nomenclature: Mesotrione; tembotrione; sweet corn, *Zea mays* L.

Key words: Cross-sensitivity, cytochrome P450, herbicide selectivity, herbicide tolerance.

Crop injury from herbicide use is an ongoing problem in sweet corn production. Sweet corn plants can be injured or, under certain circumstances, killed after POST applications of herbicides with several different modes of action, including herbicides that inhibit acetolactate synthase (ALS) (Burton et al. 1994; Robinson et al. 1994), photosystem II (Diebold et al. 2004), and 4-hydroxyphenyl-pyruvate-dioxygenase (HPPD) (Edenfield and Allen 2005; O'Sullivan et al. 2002). A wide range of responses to many POST herbicides occurs among more than 600 commercially available sweet corn hybrids and their inbred parents (Morton and Harvey 1992; O'Sullivan et al. 2002; Williams et al. 2005). Because of this variation, the adoption and use of new POST herbicides in sweet corn is limited by the potential for crop injury, particularly if comparatively little is known about the range of injury that might result from the new herbicide.

The genetic basis of sensitivity of corn to specific herbicides has been studied largely in field corn and to a lesser extent in sweet corn. Alleles at a single locus condition response to several ALS-inhibiting herbicides, including foramsulfuron (Pataky et al. 2006a), nicosulfuron (Kang 1993; Widstrom and Dowler 1995), primisulfuron (Harms et al. 1990), and rimsulfuron (Pataky et al. 2006a). Alleles at a single locus also condition response of sweet corn to carfentrazone (Pataky et al. 2006a) and mesotrione (Pataky et al. 2006a; Williams et al. 2005), although the tolerant response to mesotrione can be partially dominant and affected by herbicide dose–response relationships (Volenberg et al. 2006). Response to bentazon is conditioned by alleles at two independent loci (Bradshaw et al. 1994; Fleming et al. 1988). A single locus or very closely linked loci in the sweet corn inbred Cr1 appears to affect cross-sensitivity to all of the aforementioned herbicides (Pataky et al. 2006a), possibly by regulating the activity of one or several cytochrome P450 enzymes. Many sweet corn hybrids and inbreds that are sensitive to POST herbicides carry the same or closely linked alleles on the short arm of chromosome 5 as the herbicide-sensitive inbred Cr1 (Pataky, unpublished data). This locus or these loci appear to be the same as or very closely linked to a locus at which alleles conditioning herbicide sensitivity were previously identified as *nsf1* or *ben1* (Bradshaw et al. 1994; Kang 1993; Williams et al. 2006). The dominant allele, *Nsf1*, from a nicosulfuron-tolerant field corn inbred, B73, was recently located on the short arm of chromosome 5 among four closely linked genes with homologies to cytochrome P450 (CYP) genes (Williams et al. 2006). Nicosulfuron-sensitive inbreds GA209 and W703a, from which the *nsf1* and *ben1* alleles were identified, respectively, both contained the same 392–base pair insertion in the *Nsf1* allele relative to the tolerant inbred B73. The insertion appears to result in a nonfunctional, mutant CYP allele; thus, *nsf1* and *ben1* are the same mutant.

Different rates of metabolic inactivation are the primary basis for differential selectivity of several herbicides between crops and weeds (Barrett 1995; Grossman and Ehrhardt 2007; Mitchell et al. 2001). Differences in metabolic rates also might account for much of the variation in corn cultivar sensitivity to several herbicides. Cytochrome P450 activity in corn metabolizes herbicides in several chemical families, including all of the aforementioned herbicides; however, the number of P450 enzymes involved and regulation of their levels of activity are not clearly understood (Barrett 1995, 2000).

A mechanistic understanding of crop cultivar sensitivity to herbicides is needed so that growers, seed companies, herbicide manufacturers, and regulatory agencies can make more informed decisions about current risks of crop injury from herbicides. In the case of sweet corn, new and existing

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herbicides with the potential to injure crops are routinely evaluated on hybrids and inbreds each year; however, the high turnover of germplasm limits the prolonged usefulness of such information. An understanding of the genetics of sweet corn sensitivity to herbicides could reduce risk, in part, by helping plant breeders identify and eliminate alleles for sensitivity in germplasm used to produce commercial hybrids.

Tembotrione, an HPPD inhibitor, was recently registered for use in several types of corn, including field corn, seed corn, popcorn, and sweet corn (Anonymous 2007). Tembotrione suppresses or controls several problematic broadleaf and grass weeds that are currently difficult for corn growers to manage. However, severe injury and plant death has been reported on certain sweet corn hybrids treated with tembotrione (Anonymous 2007; Bollman et al. 2006; Edenfield and Allen 2005).

Mesotrione and tembotrione have a similar chemical structure and mode of action (Anonymous 2007; Mitchell et al. 2001). Both herbicides severely injure sweet corn hybrids Merit and Shogun (Bollman et al. 2006; Edenfield and Allen 2005; Pataky et al. 2006b). On the basis of segregation of progeny in F_2, BC_1, BC_2, BC_1S_1, BC_2S_1, $F_{2:3}$, and $F_{3:4}$ generations and tests of allelism with GA209 and W703a, mesotrione sensitivity in the sweet corn inbred Cr1 appears to be conditioned by a single allele on the short arm of chromosome 5 that is the same as or very closely linked to the CYP gene previously designated as *nsf1* and *ben1* (Pataky et al., unpublished; Pataky et al. 2006a; Williams et al. 2005). Because alleles at this locus or in this chromosome region condition responses to multiple P450-metabolized herbicides, and because mesotrione and tembotrione have similar chemical structure, we hypothesized that the genetic basis of sensitivity to tembotrione in sweet corn is the same as mesotrione and that hybrids respond similarly to the two herbicides.

Our objectives were (1) to test the hypothesis that responses to tembotrione and mesotrione are conditioned by alleles at the same or closely linked loci and (2) to test the hypothesis that the extent of herbicide injury from tembotrione and mesotrione on sweet corn hybrids is similar.

Materials and Methods

Plant Material. $F_{3:5}$ families derived from a cross of sweet corn inbreds Cr1[1] (mesotrione sensitive) and Cr2[2] (mesotrione tolerant) were used to test the hypothesis that the genetic basis of sweet corn sensitivity to tembotrione and mesotrione is conditioned by alleles at the same locus or closely linked loci. $F_{3:5}$ families were developed by self-pollinating F_1 plants of Cr1 × Cr2 on an ear-to-row basis for three generations. $F_{3:5}$ families were produced by self-pollinating a minimum of eight (and usually more than 16) F_4 plants from each family and bulking F_5 seed within F_3 families. An excess of 7,500 F_5 plants in a total of 136 F_2 families were evaluated in field experiments described below with 115 and 120 F_3 families evaluated in 2006 and 2007, respectively. Because of seed availability, 99 families were common to trials in both years and 37 families were evaluated in either 2006 or 2007.

The hypothesis that sweet corn hybrids respond similarly to tembotrione and mesotrione was tested by comparing responses of 249 hybrids obtained from every major seed company with sweet corn breeding programs in North America. This group included widely grown hybrids and experimental hybrids in the final stages of development. All three major endosperm types (e.g., sugary, sugary enhancer, and shrunken-2) and hybrids grown for fresh consumption and processing markets were represented.

Genetic Basis of Responses to Tembotrione and Mesotrione. Field experiments were grown in 2006 and 2007 at the University of Illinois Crop Sciences Research and Education Center near Urbana, IL, on Flanagan silt loam (fine, smectitic, mesic Aquic Argiudoll) soils. Fields were fertilized with 202 kg N ha^{-1}. Preplant tillage included two passes each of a field cultivator and spring-tine harrow. Early-season weeds were controlled with a PRE application of 2.2 kg atrazine ha^{-1} plus 1.8 kg *S*-metolachlor ha^{-1}. $F_{3:5}$ families were arranged in a randomized complete block design with two replicates. An experimental unit was a single 3.5-m row with 12 to 18 plants per row. Experiments were planted May 8, 2006, and May 14, 2007. Tembotrione[3] was applied at a rate of 184 g ai ha^{-1} plus 1% (v/v) crop oil concentrate (COC) and 2% (v/v) 28% urea ammonium nitrate (UAN) when sweet corn had four to five visible leaf collars. The commercial formulation of tembotrione included the safener isoxadifen-ethyl in a 2 : 1 ratio by weight, respectively (herein, collectively referred to as tembotrione). The herbicide treatment was applied with a tractor-mounted compressed air sprayer equipped with nozzles delivering 187 L ha^{-1} of water at 276 kPa. Plants were evaluated visually 1 wk after treatment (WAT) for chlorotic and bleached leaves that were symptomatic of typical injury from HPPD-inhibiting herbicides. Because injury to individual F_5 plants was $>$ 50% of the leaf area affected (present) or $<$ 5% of the leaf area affected (absent), the number of plants with and without symptoms were recorded. None of the F_5 plants exhibited intermediate responses to tembotrione (i.e., 5 to 50% leaf area affected).

F_3 families were classified as sensitive, segregating, or tolerant on the basis of the response of F_5 plants. Families with all sensitive or all tolerant F_5 plants were classified as sensitive or tolerant, respectively. In other families, a chi-square goodness of fit test was used to determine whether the number of sensitive and tolerant F_5 plants fit a 5 : 3 ratio of tolerant : sensitive plants, which would be expected if a single dominant gene conditioned response to tembotrione. In F_3 families in which F_5 seed were bulked from a minimum of eight self-pollinated F_4 plants, the ratio of 5 : 3 is based on segregation of F_4 plants within the segregating F_3 families (i.e., 25% homozygous tolerant, which produce all tolerant F_5 progeny; 25% homozygous sensitive, which produce all sensitive F_5 progeny; and 50% heterozygous, which produce 75% tolerant and 25% sensitive F_5 progeny). If the null hypothesis of a 5 : 3 ratio failed to be rejected ($P > 0.01$), families were classified as segregating. If there were more than five sensitive and five tolerant F_5 plants in families for which the chi-square test produced a probability between 0.01 and 0.001, those families also were classified as segregating. This accounts for the possibility that the ratio of tolerant : sensitive F_5 plants could have been biased by the random sample of self-pollinated F_4 plants from which F_5 seed was bulked within each family, in which case, $F_{3:5}$ families would segregate, but not necessarily in a 5 : 3 ratio. If the null hypothesis of a 5 : 3 ratio was clearly rejected ($P < 0.001$), families were classified as tolerant or sensitive depending on whether the percentage of sensitive progeny was nearest to 0

or 100%, respectively. After the $F_{3:5}$ families were classified; the number of sensitive, segregating, and tolerant families were then tested by chi-square goodness of fit to a 3 : 2 : 3 ratio that would be expected if response to tembotrione was controlled by a single gene with dominant gene action.

Previously, the same F_3 families were tested as $F_{3:4}$ and $F_{3:5}$ families for response to mesotrione in two greenhouse and two field experiments (Nordby et al. 2008). F_3 family responses to tembotrione were compared with responses to mesotrione from previous trials. A binomial test of proportions (i.e., *t* test) was used to test the hypothesis that the percentage of families with similar responses (i.e., sensitive, segregating, or tolerant) to tembotrione and mesotrione were significantly greater than 34.3%, which would be expected if response to each herbicide was conditioned by an independent dominant gene; that is, 22/64 = (3/8)(3/8) + (2/8)(2/8) + (3/8)(3/8).

Injury to Hybrids. Hybrids were evaluated for responses to tembotrione and mesotrione in six field experiments at Urbana, IL, in 2007. To test a range of environmental conditions, experiments were planted May 21, June 6, and June 22. Each planting included a separate experiment in which responses to mesotrione or tembotrione were evaluated. Each experiment consisted of two replicates of 249 hybrids arranged in a randomized complete block design, except for the mesotrione trial planted May 21, which included 10 replicates of hybrids. An experimental unit was a single 3.5-m row with 12 to 18 plants. Fields were fertilized, tilled, and treated with PRE herbicides as described above. Commercial formulations of either 210 g mesotrione[4] ha^{-1} or 184 g tembotrione ha^{-1} (two times the recommended use rates) were applied when sweet corn had four to five visible leaf collars. Adjuvants for mesotrione experiments were 1% COC and 3.6% (v/v) 28% UAN. Herbicide applications and adjuvants for tembotrione are described above. Hybrids were rated visually for the percentage of leaf area with chlorosis and bleaching 1 WAT. Hybrids that were injured also were examined 3 to 4 wk after treatment. Responses to mesotrione and tembotrione were compared for each hybrid.

Previously, several sweet corn hybrids and inbreds were characterized for their genetic predisposition to injury after applications of mesotrione and nicosulfuron on the basis of responses of F_2 progeny, progeny from testcrosses with Cr1, or both (Meyer et al. 2007; Pataky, unpublished data). Of the 249 hybrids and inbreds evaluated in our trials, 4, 22, and 42 hybrids were previously identified as homozygous sensitive, SS (i.e.,*nsf1nsf1*); heterozygous, TS (i.e., *NSF1nsf1*); or homozygous tolerant, TT (i.e., *NSF1NSF1*) on the basis of homogeneous sensitive responses to nicosulfuron and mesotrione, segregating responses, or homogeneous tolerant responses, respectively, of F_2, testcross, or both progeny. The responses of these hybrids to tembotrione and mesotrione in the 2007 trials were compared with their presumed genotype at the *Nsf1* locus.

Results and Discussion

Genetic Basis of Responses to Tembotrione and Mesotrione. Of 136 $F_{3:5}$ families evaluated for responses to tembotrione in 2006 and 2007, 44 were classified as tolerant, 42 were classified as segregating, and 50 were classified as sensitive (Table 1). Of the 44 F_3 families classified as tolerant, 38 had no sensitive F_5 plants and 5 had a single sensitive F_5 plant. One family classified as tolerant had 5 sensitive and 51 tolerant F_5 plants but was classified as tolerant because the probability of fitting a 5 : 3 ratio of tolerant : sensitive, which was expected among these F_5 plants, was < 0.001. Of the 50 $F_{3:5}$ families classified as sensitive, 30 had all sensitive F_5 plants and 13 had one or two tolerant F_5 plants. Seven families with four to seven tolerant plants, but $> 85\%$ sensitive plants, were classified as sensitive because the probability of fitting a 5 : 3 ratio of tolerant : sensitive was < 0.001. Of the 42 $F_{3:5}$ families classified as segregating for response to tembotrione, 34 had a ratio of tolerant and sensitive F_5 plants, which was not significantly different ($P > 0.01$) from the expected ratio of 5 : 3. Eight families with 10 to 22% or 63 to 69% sensitive plants also were classified as segregating, even though the probability associated with the chi-square goodness of fit test was between 0.01 and 0.001. Each of these eight families included five or more tolerant and five or more sensitive F_5 plants. A plausible explanation for the lack of fit of these families to the expected 5 : 3 ratio of tolerant : sensitive F_5 plants is that the sample of F_4 plants that were self-pollinated and bulked to produce $F_{3:5}$ families did not reflect the expected 1 : 2 : 1 ratio of segregation among F_4 plants within $F_{3:5}$ families. Hence, these families were segregating, but not in a 5 : 3 ratio.

Table 1. Comparison of F_3 family responses to tembotrione and mesotrione.

Response to mesotrione[a]	Response to tembotrione[b]			Mesotrione total[c]
	Tolerant	Segregating	Sensitive	
	No. of families			
Tolerant	43	2	0	45
Segregating	1	40	5	46
Sensitive	0	0	45	45
Tembotrione total[c]	44	42	50	

[a] Response to mesotrione from Nordby et al. (2008).
[b] Response to tembotrione on the basis of mean plant response from injury ratings taken 1 wk after treatment in field trials in 2006 and 2007.
[c] Probabilities from chi-square goodness of fit tests for an expected ratio of 3 : 2 : 3 sensitive : segregating : tolerant F_3 family responses, which would be expected if herbicide sensitivity is conditioned by a single recessive gene, were $P = 0.06$ for mesotrione and $P = 0.24$ for tembotrione.

$F_{3:5}$ families cosegregated for response to tembotrione and mesotrione (Table 1). Ninety-four percent of the families (128 of 136 families) had the same response to tembotrione and mesotrione: 43 families were tolerant, 45 families were sensitive, and 40 families were segregating for their response to both herbicides (Table 1). None of the families that were tolerant to tembotrione were sensitive to mesotrione and vice versa. On the basis of the binomial test, the percentage of families with similar responses (i.e., 94%) to tembotrione and mesotrione was significantly greater ($P < 0.001$) than the 34.1% that would be expected if response to each herbicide was conditioned by an independent dominant gene. The greatest discrepancy in responses of families to the two herbicides occurred among families that previously had been classified as segregating for response to mesotrione (Table 1). Among those 46 families, 40 were classified as segregating for response to tembotrione, 1 was classified as tolerant to tembotrione, and 5 were classified as sensitive to tembotrione. Possibly, some of these families were previously misclassified for response to mesotrione considering that, in those trials, only 96% (255 of 266) of the plants of the sensitive inbred Cr1 were classified as sensitive (Nordby et al. 2008).

Alternatively, dissimilar responses of families to these two herbicides could result from recombination of two closely linked genes that each condition response to a specific herbicide. If dissimilar responses of families are due to recombination, approximately one-eighth of the recombinants would go undetected because of segregation among F_2 plants that produce F_3 families; approximately six-eighths would be fixed for response to one herbicide (sensitive or tolerant) and segregating for response to the other herbicide; and approximately one-eighth would have opposite responses (tolerant vs. sensitive) to the two herbicides. With only eight families having dissimilar responses (i.e., potential recombinants), sample size is too small to test for whether the observed number of families fit the expected ratios of approximately one-seventh of families with opposite responses and six-sevenths of families with fixed/ segregating responses. However, because only 6% of the 136 $F_{3:5}$ families have dissimilar responses, we can conclude that linkage is relatively close (i.e., about 3 to 4% recombination during formation of gametes on F_1 and F_2 plants), if in fact responses to mesotrione and tembotrione are conditioned by alleles at different loci.

Response of the inbred Cr1 to mesotrione is conditioned by alleles at a single locus on the short arm of chromosome 5S that is the same as or very closely linked to the *Nsf1/Ben1* locus that conditions response of field corn inbreds GA209 and W703a to nicosulfuron and bentazon (Bradshaw et al. 1994; Kang 1993; Pataky et al. 2006a, unpublished; Williams et al. 2005, 2006). Because the 136 $F_{3:5}$ families derived from Cr1 × Cr2 cosegregated for response to tembotrione and mesotrione, we believe that alleles at the same locus or closely linked loci condition response to both HPPD-inhibiting herbicides.

Segregation of $F_{3:5}$ families for response to tembotrione also indicate that alleles at a single locus condition sensitivity. Of the 136 families tested, 44 were classified as tolerant, 42 were classified as segregating, and 50 were classified as sensitive for their response to tembotrione (Table 2). On the basis of chi-square goodness of fit tests, this ratio of F_3 family responses is not significantly different ($P = 0.24$) from a ratio of 3 : 2 : 3 sensitive : segregating : tolerant, which would be expected if sensitivity to tembotrione was conditioned by a single recessive allele. Results were similar within each year, with probabilities for chi-square statistics of $P = 0.14$ and $P = 0.21$ for the 115 and 120 families tested in 2006 and 2007, respectively (Table 2). Although a few reports have noted that certain sweet corn hybrids are significantly injured or killed by tembotrione (Bollman et al. 2006, Edenfield and Allen 2005), these data provide evidence of the genetic basis for that injury.

Cross-sensitivity to multiple herbicides has been reported in sweet corn. Williams et al. (2005) observed associations between responses of sweet corn hybrids, inbreds, and F_2 families (from Cr1 × Cr2) to mesotrione and nicosulfuron, leading them to hypothesize that the same recessive allele or closely linked alleles conditioned sensitivity to both herbicides. Green and Williams (2004) observed similar responses among field corn inbreds. These observations were corroborated in subsequent research on the inheritance of responses to multiple P450-metabolized herbicides (Pataky et al. 2006a). The same allele or very closely linked alleles appeared to be primarily responsible for sensitivity of the inbred Cr1 to seven P450-metabolized herbicides with four unique modes of action (Nordby et al. 2008; Pataky et al. 2006a). Cytochrome P450 activity in corn metabolizes herbicides in at least six chemical families (Barrett 1995, 2000). Differential rates of metabolic inactivation are the primary basis for differential sensitivity in corn to several sulfonylurea herbicides (Barrett 1995; Fonné-Pfister et al. 1990). Our data support the hypothesis that the allele that conditions sensitivity to mesotrione in the inbred Cr1 or an allele at a very closely linked locus also conditions sensitivity to tembotrione, probably as a result of reduced cytochrome P450 activity.

Table 2. Number of F_3 families of sweet corn with tolerant, segregating, and sensitive responses to tembotrione from field trials in 2006 and 2007.

Year	n[a]	Response to tembotrione[b]			Probability[c]
		Tolerant	Segregating	Sensitive	
		No. of families			
2006	115	39	38	38	0.14
2007	120	37	37	46	0.21
Combined	136	44	42	50	0.24

[a] Total number of families.
[b] Classification of family response to tembotrione on the basis of mean plant response from injury ratings taken 1 wk after treatment.
[c] Probability of the chi-square goodness of fit test if the null hypothesis of a 3 : 2 : 3 sensitive : segregating : tolerant F_3 family response ratio, which would be expected if herbicide sensitivity were conditioned by a single recessive gene, is true.

Injury to Hybrids. One week after treatment, injury to sweet corn hybrids from tembotrione was either severe (> 50% leaf area affected) or absent; whereas injury from mesotrione 1 WAT ranged from low (≤ 10% leaf area affected) to intermediate (> 10 to 50% leaf area affected) to severe (> 50% leaf area affected). Sweet corn hybrids that were sensitive (> 50%) or tolerant (≤ 10%) responded similarly to tembotrione and mesotrione 1 WAT; however, hybrids with intermediate (> 10% to 50%) responses to mesotrione had tolerant responses to tembotrione. Seven sweet corn hybrids were severely injured by tembotrione and mesotrione, and 193 sweet corn hybrids were tolerant of tembotrione and mesotrione. No visual symptoms of tembotrione injury were observed 1 WAT on 49 hybrids that had > 10 to 50% injury from mesotrione.

Injury 7 d after application of tembotrione or mesotrione ranged from 56 to 100% on seven sensitive hybrids, including Merit, DMC 20-38, Shogun, 177A, HMX 6386 S, 0873 5623, and XTH 3175 (Table 3). Merit and Shogun exhibited severe symptoms of injury from tembotrione applications in previous trials (Bollman et al. 2006; Edenfield and Allen 2005). Three weeks after treatment with mesotrione, newly emerged leaf tissue was green, and the seven sensitive hybrids appeared to have resumed normal growth. Conversely, 3 to 4 WAT with tembotrione, most plants of these seven hybrids were dead or chlorotic, stunted, and nearly dead. Thus, the most sensitive hybrids in our trials were injured more severely by tembotrione than by mesotrione in all three plantings. Conversely, hybrids and inbreds with intermediate responses to mesotrione had no visual symptoms of injury from tembotrione (Table 3).

Responses of hybrids to tembotrione and mesotrione shared a distinct pattern for hybrids that previously were identified as being homozygous sensitive (SS), homozygous tolerant (TT), or heterozygous (TS) for CYP alleles on chromosome 5S conditioning sensitivity to P450-metabolized herbicides (Table 4). The four hybrids that were homozygous for alleles conditioning sensitivity had > 50% injury 1 WAT with

Table 3. Sweet corn hybrids with sensitive responses to tembotrione and mesotrione or intermediate responses to mesotrione applied POST in six field experiments in 2007.

Hybrid	Endosperm type[a]	Kernel color[b]	Seed source[c]	Response to mesotrione	Response to tembotrione
				Mean % injury 1 WAT	
Merit[d]	su	Y	Sem	91	88
DM 20-38[d]	su	Y	DM	87	87
Shogun[d]	sh2	Y	Cr	81	80
177A[d]	sh2	Y	IFS	74	50
HMX 6386 S	sh2	Y	HM	73	63
0873 5623	sesu	Y	Sem	60	52
XTH 3175	sh2	W	IFS	51	57
Hollywood[e]	sh2	B	Sem	44	0
Experimental 25	sh2	Y	Rog	40	0
Supersweet Jubilee Plus[e]	sh2	Y	Rog	39	0
WSS 1262	sh2	W	Rog	36	0
Basin R[e]	sh2	Y	Sem	35	0
Columbus[e]	sh2	Y	SnRv	34	0
0876 5391	sh2	B	Sem	32	0
HMX 7389 S	sh2	Y	HM	32	0
HMX 7390 S	sh2	Y	HM	29	0
Sure Gold[b]	sh2	Y	HM	29	0
Even Sweeter	sh2	W	Sem	27	0
GG Code 193	su	Y	GG	26	0
HW 2545 OM	sh2	W	SnRv	26	0
0875 6476	sh2	Y	Sem	26	0
Devotion[e]	sh2	W	Sem	26	0
Experimental 23	su	Y	Rog	26	0
GSS 6550	sh2	Y	Rog	26	0
HB 2162 OL	sh2	B	SnRv	24	0
EX 0873 7009	sh2	B	Sem	24	0
Supersweet Jubilee[e]	sh2	Y	Rog	23	0
EX 0874 6057	sh2	B	Sem	22	0
EX 0843 4712	sh2	B	Sem	21	0
WH 1163	sesu	W	Rog	20	0
Max[e]	sh2	Y	HM	19	0
XTH 3173	sh2	W	IFS	19	0
El Toro[e]	sesu	Y	Sem	18	0
EX 0832 4148	su	Y	Sem	18	0
Experimental 24	se	Y	Rog	18	0
Jubilee[e]	su	Y	Rog	17	0
Ranger[e]	sh2	Y	HM	17	0
Summer Sweet 781 Ultra	sh2su	W	AC	16	0
Mirai 302 BC	sh2	B	Cent	16	0
Symmetry	sh2	W	Cr	16	0
EX 0874 5857	sh2	B	Sem	15	0
GH 6377	su	Y	Rog	15	0
Denali	se	W	MM	14	0
EX 0874 6106	sh2	Y	Sem	14	0
BC 503	sh2	Y	Bas	14	0
Accord[e]	se	B	MM	13	0
Empire[e]	su	Y	SnRv	13	0
GH 6198	su	Y	Rog	13	0
HMX 7388	su	Y	HM	13	0
277A[e]	sh2	B	IFS	12	0
HW 1336 OK	sh2	W	SnRv	12	0
0874 5040	sh2	Y	Sem	11	0
179A	sh2	Y	IFS	11	0
Exp. Y 30748	se	Y	MM	11	0
Mirai 336 BC	sh2	B	Cent	11	0
Precious Gem[c]	se	B	MM	11	0

[a] Endosperm type: se, sugary enhancer; sh2, shrunken-2; su, sugary.
[b] Kernel color: B, bicolored; W, white; Y, yellow.
[c] Seed source: AC, Abbott & Cobb Inc., Feasterville, PA; Bas, Basso, Buenos Aires, Argentina; Cen, Centest, Harvard, IL; Cr, Crookham Company, Caldwell, ID; DM, Del Monte, Mendota, IL; GG, Green Giant, LeSueur, MN; HM, Harris Moran Seed Company, Modesto, CA; IFS, Illinois Foundation Seeds Inc., Champaign, IL; MM, Mesa Maize, Olathe, CO; Rog, Syngenta Seeds Inc. Rogers Brands, Boise, ID; Sem, Seminis Inc., Oxnard, CA; SnRv, Snowy River Seeds, Orbost, Australia.
[d] Hybrids identified previously as homozygous for alleles (e.g., *nsf1nsf1*) conditioning sensitivity to the P450-metabolized herbicides nicosulfuron and mesotrione.
[e] Hybrids identified previously as heterozygous for alleles (e.g., *NSF1nsf1*) conditioning sensitivity to the P450-metabolized herbicides nicosulfuron and mesotrione.

mesotrione and tembotrione in 2007 and thus were classified as sensitive (Table 4). By 3 to 4 WAT, these four hybrids had resumed normal growth if treated with mesotrione, but they were dead or nearly dead if treated with tembotrione. All of the hybrids that were homozygous for alleles conditioning tolerance had no visual injury from applications of tembotrione, and 41 of those 42 hybrids had $< 10\%$ injury from mesotrione. The lone exception had 13% injury 1 WAT with mesotrione.

Table 4. Response to mesotrione and tembotrione[a] in 2007 field experiments for 68 sweet corn hybrids previously identified as homozygous for alleles conditioning sensitivity (SS) to nicosulfuron and mesotrione, homozygous for alleles conditioning tolerance (TT) to these two herbicides, or heterozygous (TS) for a sensitive and tolerant allele.

		Response to mesotrione			Response to tembotrione		
		% Injury					
Genotype[b]	*n*[c]	> 50	> 10–50	≤ 10	> 50	> 10–50	≤ 10
		No. of hybrids					
SS	4	4	0	0	4	0	0
TS	22	0	15	7	0	0	22
TT	42	0	1	41	0	0	42

[a] Response to tembotrione and mesotrione on the basis of mean plant response from injury ratings taken 1 wk after treatment in six field trials in 2007.
[b] Hybrids identified previously as homozygous for alleles conditioning sensitivity (SS), heterozygous (TS), and homozygous for alleles conditioning tolerance (TT) on the basis of response of F_2 and testcross progeny after applications of mesotrione and nicosulfuron, from Meyer et al. (2007) and Pataky (unpublished data).
[c] Total number of hybrids with known genotypes.

The primary difference 1 WAT between responses of hybrids to tembotrione and mesotrione occurred among hybrids that were heterozygous for alleles conditioning sensitivity and tolerance. None of the 22 heterozygous hybrids were injured by tembotrione, but 15 of the 22 had an intermediate response (10 to 50% injury) to mesotrione.

Because cosegregation of $F_{3:5}$ families indicated that the same or closely linked alleles condition responses to tembotrione and mesotrione, differences in responses of heterozygous hybrids to these two herbicides indicated that other factors also are affecting responses. Using hybrids nearly isogenic for a CYP gene on chromosome 5S in dose–response assays, Volenberg et al. (2006) reported that following applications of mesotrione in growth chamber studies, biomass of the heterozygous version of the near-isogenic hybrid was intermediate to that of the homozygous tolerant and homozygous sensitive versions of the isogenic hybrid. Conversely, in recent field experiments, no injury or differences in yield were observed between homozygous tolerant and heterozygous versions of five near-isogenic hybrids after applications of tembotrione at rates of 46, 92, 184, 368, or 736 g ai ha^{-1}, i.e., 0.5 to 8 times recommended use rates (Pataky and Williams, unpublished data). Nevertheless, the homozygous sensitive versions of all five near-isogenic hybrids were killed or severely stunted at all five rates of tembotrione. Perhaps the safener isoxadifen-ethyl, which was formulated with tembotrione, reduced injury to heterozygous hybrids by stimulating herbicide metabolism by cytochrome P450 enzymes, whereas the safener had little effect on homozygous sensitive plants. Bunting et al. (2004) found that isoxadifen-ethyl increased foramsulfuron absorption, translocation, metabolism, and crop tolerance in a foramsulfuron-sensitive field corn hybrid; however, the genetic condition of the hybrid at the CYP locus on chromosome 5S was not known. Additional research is necessary to fully understand variable responses of hybrids heterozygous for alleles that appear to be primarily responsible for conditioning sensitivity to tembotrione and mesotrione and to determine the effects of isoxadifen-ethyl in relation to the genetic condition of the hybrid.

Our research demonstrated that $F_{3:5}$ families from a cross of a mesotrione-sensitive (Cr1) and a mesotrione-tolerant (Cr2) inbred cosegregated for response to mesotrione and tembotrione. Sensitivity to mesotrione in Cr1 is conditioned by a single allele on the short arm of chromosome 5 that appears to be the same as or very closely linked to a CYP allele previously identified as *nsf1* or *ben1* (Bradshaw et al. 1994; Kang 1993; Pataky et al. 2006a, unpublished; Williams et al. 2005, 2006). This allele or closely linked alleles condition cross-sensitivity to multiple P450-metabolized herbicides (Barrett 1995, 2000; Nordby et al. 2008; Williams et al. 2005), including, on the basis of the results of this study, tembotrione. Consequently, 193 sweet corn hybrids that were tolerant to mesotrione also were tolerant to tembotrione. However, despite the common genetic basis for response to mesotrione and tembotrione, hybrids with sensitive or intermediate responses to mesotrione did not have identical responses to tembotrione. At 1 WAT, sensitive hybrids were severely injured by both herbicides, but by 3 to 4 WAT, sensitive hybrids treated with mesotrione had apparently resumed normal growth, whereas those treated with tembotrione where dead or nearly dead. Conversely, at 1 WAT, hybrids with intermediate levels of injury (> 10 to 50%) after applications of mesotrione had no visual symptoms of injury from applications of tembotrione. Therefore, 242 of the 249 hybrids tested were highly tolerant of tembotrione under conditions of these experiments; however, severe injury, including plant death, resulted from tembotrione application to the seven hybrids that were presumably homozygous for alleles conditioning sensitivity.

Sources of Materials

[1] Cr1 sweet corn inbred, Crookham Company, Caldwell, ID.
[2] Cr2 sweet corn inbred, Crookham Company, Caldwell, ID.
[3] Tembotrione, Laudis® herbicide, Bayer CropSciences, Research Triangle Park, NC.
[4] Mesotrione, Callisto® herbicide, Syngenta, Greensboro, NC.

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Literature Cited

Anonymous. 2007. Laudis Technical Bulletin. Research Triangle Park, NC: Bayer Cropscience. 6 p.

Barrett, M. 1995. Metabolism of herbicides by cytochrome P450. Drug Metab. Drug Interact. 12:299–315.
Barrett, M. 2000. The role of cytochrome P450 enzymes in herbicide metabolism. Pages 25–37 *in* A. H. Cobb and R. C. Kirkwood, eds. Herbicides and Their Mechanisms of Action. Boca Raton, FL: CRC.
Bollman, J. D., C. M. Boerboom, and R. L. Becker. 2006. Efficacy and tolerance of HPPD-inhibiting herbicides in sweet corn. N. Cent. Weed Sci. Soc. Abstr. 61:131.
Bradshaw, L. D., M. Barrett, and C. G. Poneleit. 1994. Inheritance of bentazon susceptibility in a corn (*Zea mays*) line. Weed Sci. 42:641–647.
Bunting, J. A., C. L. Sprague, and D. E. Riechers. 2004. Physiological basis for tolerance of corn hybrids to foramsulfuron. Weed Sci. 52:711–717.
Burton, J. D., E. P. Maness, D. W. Monks, and R. R. Robinson. 1994. Sulfonylurea selectivity and safener activity in 'Landmark' and 'Merit' sweet corn. Pesticide Biochem. Physiol. 48:163–172.
Diebold, S., D. Robinson, J. Zandstra, J. O'Sullivan, and P. H. Sikkema. 2004. Sweet corn sensitivity to bentazon. Weed Technol. 18:982–987.
Edenfield, M. and J. Allen. 2005. Sweet corn and popcorn tolerance to tembotrione. N. Cent. Weed Sci. Soc. Abstr. 60:91.
Fleming, A. A., P. A. Banks, and J. G. Legg. 1988. Differential response of maize inbreds to bentazon and other herbicides. Can. J. Plant Sci. 68:501–507.
Fonné-Pfister, R., J. Gaudin, K. Kreuz, K. Ramsteiner, and E. Ebert. 1990. Hydroxylation of primisulfuron by an inducible cytochrome P450-dependent monooxygenase system from maize. Pestic. Biochem. Physiol. 37:165–173.
Green, J. M. and M. E. Williams. 2004. Correlation of corn (*Zea mays*) inbred response to nicosulfuron and mesotione. Weed Sci. Soc. Am. Abstr. 44:4.
Grossman, K. and T. Ehrhardt. 2007. On the mechanism of action and selectivity of the corn herbicide topramezone: a new inhibitor of 4-hydroxyphenylpyruvate dioxygenase. Pest Manag. Sci. 63:429–439.
Harms, C. T., A. L. Montoya, L. S. Privalle, and R. W. Briggs. 1990. Genetic and biochemical characterization of corn inbred lines tolerant to the sulfonylurea herbicide primisulfuron. Theor. Appl. Genet. 80:353–358.
Kang, M. S. 1993. Inheritance of susceptibility to nicosulfuron herbicide in maize. J. Hered. 84:216–217.
Meyer, M. D., J. K. Pataky, J. D. Bollman, C. M. Boerboom, and M. M. Williams, II. 2007. Genetic basis for varied responses of sweet corn hybrids to three cytochrome P450-metabolized herbicides in multi-state trials. Am. Soc. Agron. Abstr. http://a-c-s.confex.com/crops/2007am/techprogram/P33748.htm. Accessed: February 28, 2008.
Mitchell, G., D. W. Bartlett, T.E.M. Fraser, T. R. Hawkes, D. C. Holt, J. K. Townson, and R. A. Wichert. 2001. Mesotrione: a new selective herbicide for use in maize. Pest Manag. Sci. 57:120–128.
Morton, C. A. and R. G. Harvey. 1992. Sweet corn (*Zea mays*) hybrid tolerance to nicosulfuron. Weed Technol. 6:91–96.
Nordby, J. N., M. M. Williams, II, J. K. Pataky, D. E. Riechers, and J. D. Lutz. 2008. A common genetic basis in the sweet corn inbred Cr1 for cross-sensitivity to multiple cytochrome P450-metabolized herbicides. Weed Sci. 56:376–382.
O'Sullivan, J., J. Zandstra, and P. Sikkema. 2002. Sweet corn (*Zea mays*) cultivar sensitivity to mesotrione. Weed Technol. 16:421–425.
Pataky, J. K., J. N. Nordby, M. M. Williams, II., and D. E. Riechers. 2006a. Inheritance of cross-sensitivity in sweet corn to herbicides applied postemergence. J. Am. Soc. Hortic. Sci. 131:744–751.
Pataky, J. K., M. M. Williams, II., B. Warsaw, M. Meyer, and J. Moody. 2006b. Sweet Corn Hybrid Disease Nursery—2006. Midwestern Vegetable Variety Trial Report for 2006, Purdue University, AES Bulletin B18048, Pp. 59–74.
Robinson, D. K., D. W. Monks, and J. R. Schultheis. 1994. Effect of nicosulfuron applied postemergence and post-directed on sweet corn (*Zea mays*) tolerance. Weed Technol. 8:630–634.
Volenberg, D. S., M. M. Williams, II., J. K. Pataky, and D. E. Riechers. 2006. Responses of tolerant and sensitive sweet corn inbreds and near isogenic hybrids to postemergence herbicides with different modes of action. N. Cent. Weed Sci. Soc. Abstr. 61:213.
Widstrom, N. W. and C. C. Dowler. 1995. Sensitivity of selected field corn inbreds (*Zea mays*) to nicosulfuron. Weed Technol. 9:779–782.
Williams, M., S. Sowinski, T. Dam, and B. L. Li. 2006. Map-based cloning of the *nsf1* gene of maize. Page 49 *in* Program and Abstracts of the 48th Maize Genetics Conference. [Abstract].
Williams, M. M., II., J. K. Pataky, J. N. Nordby, D. E. Riechers, C. L. Sprague, and J. B. Masiunas. 2005. Cross-sensitivity in sweet corn to nicosulfuron and mesotrione applied postemergence. HortScience 40:1801–1805.

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